

## HETEROPLASTIC TRANSPLANTATION AND SPECIES SPECIFICITY

### I. A COMPARISON OF THE EFFECTS OF RECIPROCAL CHORIO-ALLANTOIC TRANSPLANTS OF MACERATED AND UNMACERATED DUCK AND CHICK KIDNEY TISSUE

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Heteroplastic transplantations, especially those designed to study the development of species specificity in the embryo, have often led to results of doubtful significance. Frequent contradictions appeared, and interpretations resulted in ambiguities. As a consequence, any correlation between the transplantability of a tissue and species specificity has been questioned. To study this, and contingent problems, a series of transplantation experiments has been planned using hosts and donors of a stage in development when the relation in question first becomes apparent, and presumably, therefore, when it can be most easily analyzed.

In investigating some of the various aspects of heteroplastic transplantations, Sandstrom and Kauer (1933a) studied the host reactions incited by macerated duck kidney tissue implanted on the chorio-allantoic membrane of the chick. They discovered that macerated tissue from the early embryo, e.g., 13 days of incubation, could grow and differentiate, and to a certain degree reconstitute itself into what appeared to be an integrated kidney tissue, unhampered by, and without effect on, the host. But as the donor embryo approached the hatching stage, not only did the macerated kidney tissue fail to grow, but there was evidence that it had a lethal effect on the host. While this drastic effect might have developed gradually, it expressed itself rather suddenly at hatching. Accordingly, implantations of macerated kidney tissue from just-hatched ducklings caused a mortality rate of 100 per cent. They attributed the death of the hosts to an anaphylactoid shock brought about by certain species specific qualities which the protoplasm of the donor tissue apparently acquired just previous to the time of hatching. The results were so striking that a more complete investigation, with an attempt to relate the findings to the results of experiments involving intact tissue transplants, seemed desirable.

A small portion of the results was reported in abstract by Sandstrom, Eisen, and Siffert (1939).

#### EXPERIMENTAL PROCEDURE

Two series of heteroplastic transplantations were made, namely, duck to chick, and chick to duck. In addition, homoplastic transplants were made to both the chick and the duck. Single-combed White Leghorn chickens and White Pekin ducks furnished both donors and hosts. The ages of the chick donors were selected as follows: embryos of 16 and 20 days' incubation, 5-day old chicks, and adults of uncertain age, while those of the duck donors were embryos of 13, 21, 24, and 27 days' incubation, and ducklings, 1, 4, and 7 days old. The chick hosts were embryos of 9 days' incubation, and the duck hosts, of 13 days' incubation.

In all of the transplantations, tissue from the metanephric kidneys was used. The organ was dissected free from adjacent tissues, and kept in normal saline solution at 38° to 39° C. until a sufficient number of metanephroi of any one stage had been obtained. The time required to collect them varied according to the age of the donor, but usually, at most, it was a matter of only a few minutes. The metanephroi were then completely macerated in a small quantity of saline solution by grinding with a pestle in a glass mortar. After this vigorous treatment the contents of the mortar were washed into a centrifuge tube with saline solution, and the ground tissue centrifuged out. By the technique characteristic of chorio-allantoic grafting, a bit of the collected tissue, only 1 cubic millimeter, was implanted on the chorio-allantoic membrane of the host which was then returned to the incubator for continued incubation.

Examinations of the hosts were made at set intervals in various ways. Usually they were examined at some interval within 48 hours after the implantation, either by candling, noting the condition of the inner shell membrane through an opening into the air chamber, or opening the egg and sacrificing the embryo if it were still alive. In some cases the embryos were to be utilized for another type of experiment, and only a few eggs were examined at any one time, so that the observations extended over several days. Whenever such variations in the procedure might have some bearing on the interpretations of the results, they will be mentioned specifically.

In comparing the results obtained after the implantation of macerated, with those of intact tissue, data from other experiments were utilized for the latter. For this reason the ages of the donors of the intact tissue do not always agree with those of the macerated tissue. The deviation, however, is not so great as to offer any serious objections.

Perhaps it is well to point out here that the mortality rate of the developing chick hosts, which forms the basis of this report, might readily be affected by many factors, some of which are undoubtedly the result of operative procedure, although there is reason to believe that the latter is not of considerable consequence. There are in addition some factors which, under ordinary circumstances, are beyond the control of the experimenter. Reference is being made, of course, to the hardness of the poultry stock, the handling of the eggs, weather conditions in transit, etc. While a check had been made with respect to the source of the eggs, yet there have been instances when comparable ex-

TABLE I

*Mortality rate following implantation of macerated kidney tissue*

Host-donor relationship	Number of cases	Age of donor	Age of host	Length of time on host	Number dead	Mortality rate
		(in days)	(in days)	(in days)		per cent
Duck-on-chick	14	13	9	2	3	21.4
	56	21	9	2	21	37.5
	28	24	9	1.25	7	25.0
	27	27	9	2	16	59.2
	57	28	9	1.5	50	87.7
	19	32	9	2	19	100.0
	103	35	9	2	103	100.0
Chick-on-duck	31	16	13	2 to 9	14	45.1
	27	20	13	2	14	51.8
	63	26	13	2	33	52.3
	29	Adult	13	2	8	27.5
Duck-on-duck	39	35	13	2	11	28.2
Chick-on-chick	38	26	9	2	9	23.7

perimental treatment was afforded two separate shipments of eggs, but with quite contrasting results. Although no results were considered when there was reason to suspect unfavorable treatment of the eggs that might affect the viability of the embryos before incubation, e.g., sudden extreme changes in temperature while the eggs were in transit from the hatchery to the laboratory, nevertheless, the percentages which express the mortality rate will show, particularly when the numbers are low, considerable variation which must pass unexplained.

## RESULTS

The results of the heteroplastic transplantations are summarized in Table I. Perusal of the table reveals that the macerated duck tissue

had little effect on the chick hosts until the age of the donor material approached that of the hatching period, i.e., the twenty-seventh day of incubation. At this time, the day before hatching, the mortality rate among the hosts, resulting from the implantation of the macerated duck tissue, increased to 59.2 per cent. When the macerated tissue was obtained from donors on the day of hatching, there was an increase in the death rate following implantation to 87.7 per cent, and from older stages of donors it was consistently 100.0 per cent. All the deaths occurred within 48 hours from the time of the implantation. It is an escapable fact that some of the embryos died from causes unrelated to the experiment, but the marked effect on the hosts produced by tissue obtained from donors of hatching age, or older, was most decidedly demonstrated.

In the hosts which survived long enough to permit incorporation, a gross and microscopical study of the chorio-allantoic membrane bearing the implanted tissue indicated some rather definite changes from the normal. Most pronounced was the packing, and apparent agglutination, of the erythrocytes in the blood vessels, both arteries and veins. These vessels in some instances were distended as though by an internal pressure. Diffuse blood clots not infrequently marked the area of implantation, and hemorrhages were also found in the embryo itself. The hemorrhages were for the most part subcutaneous, but some were localized in the hind brain.

While the heteroplastic transplantations of macerated duck kidney tissue displayed certain well-defined effects on the chick hosts, the reciprocal relations did not. As indicated in Table I, the macerated metanephric tissue from the chick, regardless of its age, when implanted on the chorio-allantoic membrane of the duck, had no significant effect on the hosts. To be sure, the death rate for the 16-day donor was not actually as great as indicated, for, as given, the percentage includes all of the deaths that occurred through a period extending from 48 hours to 9 days. While it is possible that the mortality rate at the end of the second day could have been equal to that given in the table, this was not true because the degree of development of some of the host embryos was greater than that of 11 days. The significance, however, of what thus might have been interpreted as an increase in the mortality, is immediately dissipated by the pronounced decrease following the implantation of tissue from the adult chicken. Further evidence that no reaction comparable to that resulting from the implantation of macerated duck tissue occurred, was corroborated by a histological examination of the host membrane. It showed no packing of the blood vessels, nor were diffuse blood clots present in the membrane to the extent that they

were in the other group of heteroplastic implantations. Likewise, brain and subcutaneous hemorrhages were lacking.

The two series of homoplastic transplants involving hatched chicks and ducklings indicated no effects on the hosts. The results, as summarized in Table I, compare favorably with those of the chick-on-duck implantations, and duck-on-chick before the donor had approached the hatching stage.

TABLE II

*Mortality rate following implantation of intact kidney tissue*

Host-donor relationship	Number of cases	Age of donor	Age of host	Length of time on host	Number dead	Mortality rate
		(in days)	(in days)	(in days)		per cent
Duck-on-chick	84	9	9	2 to 9	32	38.1
	94	13	9	2 to 9	46	48.9
	22	19	9	2 to 9	12	54.5
	19	24	9	2 to 9	13	68.4
	43	27	9	2 to 9	16	37.2
	39	Adult	9	2 to 9	4	10.3
Chick-on-duck	41	9	14	2 to 11	6	14.6
	24	13	14	2 to 10	3	12.5
	42	19	14	2 to 10	3	7.1
	16	24	14	2 to 10	9	56.2
	28	27	14	2 to 10	3	10.7
	39	Adult	14	2 to 9	13	33.3
Duck-on-duck	76	13	13 to 14	2 to 10	30	39.4
	36	21	13	2 to 10	10	27.7
	17	24	13	2 to 10	7	41.1
	14	27	13	2 to 10	7	50.0
	27	32	14	2 to 10	9	33.3
Chick-on-chick	62	9	9	2 to 9	18	29.0
	41	19	9	2 to 9	16	39.0
	55	24	9	2 to 9	24	43.6

The effects of the heteroplastic transplantations of the intact, un-macerated kidney tissue are summarized in Table II. The data were compiled from many experiments performed over a period of years, but the general experimental conditions were precisely the same as for the implantations of macerated tissue. The mortality rate, as expressed in this table, however, is not that present after 48 hours, but the total after various periods extending from 48 hours to 9 days. Since many of the hosts showed development beyond the 11-day stage, the percentage that might have expressed the mortality rate at the end of 48 hours can always be assumed to be somewhat less than that recorded. Examina-

tion of the mortality rate shows no significant differences between the various age levels. The same is true for the reciprocal series of chick-on-duck implants. Both sets in the heteroplastic series compare favorably with those in the homoplastic.

#### DISCUSSION

The results of the implantation of macerated duck tissue to the chick membrane are significant for an understanding of many problems involved in the development of species specificity in the embryo. They emphasize the virtually complete independence of those factors governing the transplantability of a tissue and certain intracellular factors which may be responsible for species specificity, and which become effective only when cell boundaries are broken down. This fact becomes more apparent by a comparison of the effects that macerated duck kidney tissue had on the chick hosts, as included in Table I, with the effects of comparable intact tissue, as summarized in Table II. In the latter group there was no increase in the percentage of deaths following the implantation of tissue from donors of the hatching age, or older. The intracellular factors which were responsible for the effects produced with macerated tissue, although present, were confined within the cell boundaries, and could not, therefore, demonstrate their presence. Furthermore, a comparison of the duck-on-chick transplants of macerated tissue with homoplastic transplants from comparable donors, suggests that the effects on the hosts may be due to factors of importance in species specificity.

Pursuant to the above, it is clear that the problems related to the development of species specificity cannot be adequately analyzed by the utilization of intact tissue transplants only. There is no question that the reaction incited in the tissues of the host by the presence of a bit of engrafted tissue is a response to metabolic substances of a toxic nature, as postulated by Loeb (1930). In other words, it is a typical, localized inflammation reaction. While it is conceivable that a relation exists between the kind and intensity of such reactions and the genetic relationship between the donor and recipient, as contended by Loeb (*loc. cit.*), and further substantiated by Loeb and King (1935), modifying factors are so numerous that they tend to obscure, or confuse the real expression of these relations. For example, Sandstrom (1932) obtained necrosis of intact metanephric tissue of the duck in chorio-allantoic grafts on the chick. The necrosis was preceded and accompanied by a typical cellular reaction. On the other hand, Sandstrom and Kauer (1933*b*) found that cartilage from donors of the same species and of comparable, or older stages, displayed no necrosis, and little

cellular reaction from the chick membrane. Although the species specificity factor was unchanged in the two series of transplants, and therefore should have called forth similar reactions, such was not the case. The nature and greater intensity of the reaction against the actively functional metanephric implant was presumably due to a greater production of toxic substances which produce a proportionately greater reaction in the surrounding host tissue. This response interfered with complete incorporation and vascularization, and ultimately led to an absorption of the transplant and its replacement by connective tissue. In contrast, the passively functional cartilage produced relatively little toxin, and the incorporation of the avascular tissue, rapidly accomplished by the mere contact with the host connective tissue, was unhampered by any reaction which might be antagonistic to a foreign tissue.

That the local reaction can be modified in other ways was also demonstrated by Sandstrom (1934), who obtained functional duck kidney tissue equivalent to that of a 2-day duckling from the chorio-allantoic membrane of the chick after it had grown thereon for a period of 11 days. This condition was possible only because of ideal circumstances wherein the implant was properly incorporated and vascularized before local reactions could interfere. As a result, the toxins of the transplant were removed by the functional graft itself. There was, under these circumstances, no evidence of a host reaction. The most ideal conditions for the establishment of a functional transplant which might grow unhampered by the host would be the concomitant growth and differentiation of the transplant and host. Such an ideal was most successfully achieved by Milford (in press), who obtained normal functional duck kidney tissue equivalent in age to 35 days' incubation after having grown as an intracoelomic graft in the chick for a period of 18 days. The storage of excretory products in these instances offers no difficulty because of the characteristic nature of the excretory function in the bird where uric acid is deposited in the cavity of the allantois after the reabsorption of water by the allantoic membrane. The amount of concentrated uric acid produced by a small graft of kidney tissue after the removal of the water would be relatively small, and readily stored in tissue spaces within the host membrane. As pictured by Sandstrom (*loc. cit.*), it is possible for large kidney tissue grafts to be connected directly by means of their tubules to large vesicular-like spaces which facilitates waste removal. Inasmuch as these and many other factors, will modify the local inflammation reaction, considerable care must be exercised in using transplantations for the study of species specificity, and the resulting conclusions, therefore, must of necessity, be very general.

The violent reaction in the chick host caused by the macerated duck tissue was not a localized manifestation, but was distributed widely in the embryonic system through the blood vascular system. The microscopic and gross examinations of the hosts and their membranes revealed pronounced effects on the blood vascular system not only in the area of the implant, but also in the embryo itself. It could not, of course, be ascertained whether these visible effects were primary or secondary, but the general indications were that the implanted material contained substances which caused an agglutination of the blood cells, and led ultimately to the collapse of the respiratory system. Death was therefore presumably the result of suffocation. It is impossible to state whether this phenomenon of agglutination is in any way related to an immunological reaction, perhaps, in some manner, to the development of antigenic properties and antibody production in the developing embryonic donor and host respectively. Only preliminary attempts have been made to determine quantitatively the minimum amount of macerated tissue necessary to produce death. The effective quantity must be very small, because, of the 1 cu. mm. of macerated tissue implanted, only a small part comes in immediate contact with the membrane. Furthermore, in a few preliminary experiments, it has been found that as little as .3 cc. (5 drops) of the unfiltered, supernatant fluid left after centrifuging macerated kidney tissue from a duckling 4 days old, was sufficient to kill the host in less than 30 hours, but none died before 8 hours. In the transplantation of intact kidney tissue pieces of the metanephros were implanted because the entire organ was too large for proper incorporation. It follows that some of the cells must have been injured by cutting, and the question arises whether the release of the intracellular substance, since the effective dose is so small, did not in some manner bear on the transplantability of the intact tissue. Since care was taken to cause a minimum amount of injury, the amount of intracellular substance freed apparently was insufficient to have demonstrable results, although a comparison of the duck-on-chick with the chick-on-duck transplantations of intact tissue (see Table II) reveals in general a lower mortality rate for the latter group than the former, thus simulating the effects of macerated implants. The significance of this difference is questionable, particularly since the percentages are higher in all age groups, and kidney tissue from duck embryos of 27 days' incubation, and even adults, caused no greater percentage of deaths than did the implants from younger duck donors.

While the results of the implantation of the macerated duck kidney tissue to the chorio-allantoic membrane of the chick have been the source of many interesting speculations and additional problems, the implants

of the macerated chick tissue to the membrane of the duck had no effect on the hosts. Such a complete lack of reciprocity is not without precedence, and in this instance was not wholly unexpected. Sandstrom (1936) demonstrated a decided difference between the results of reciprocal chorio-allantoic transplantations of intact metanephric tissue of the duck and chick. A comparison made between the two types of reciprocal implants, i.e., intact and macerated, tends to confound rather than clarify the problems involved. Whereas the macerated duck tissue at certain ages had profound effects on the chick host, the intact tissue of comparable ages, and even from adults, was rapidly incorporated. In the reciprocal host-donor relationship, in which the macerated tissue had no effect, the intact tissue was only slowly incorporated. No explanation is attempted for these seemingly paradoxical results, but they do give emphasis to the contention that transplantability is independent of intracellular properties.

A comparison of the results of the transplantation of intact and macerated tissue of the duck to the embryonic chick host also displays a striking parallelism to the results of experiments which have attempted to correlate transplantability with serological blood properties of the host. Several workers have compared the success of skin grafts to serological relationships. Baldwin (1920) and Kubanyi (1924) support the conclusions of Masson (1918), who seems to have demonstrated that skin implants could be successful only if the donor was of the same blood group as the host. Others, particularly Kozelka (1933), found evidence to the contrary. He made detailed investigations of the problem, transplanting such integumentary structures of the chicken as the wattle, comb, and spur. He found no relation between the success of the transplant and the agglutinating phenomenon of the host's blood. In the assumed parallelism, the integumentary grafts of Kozelka can be compared to the intact kidney tissue. Any apparently antagonistic reaction would be local, and a response of the host's tissue to the metabolic toxins given off by the transplant. It would therefore be independent, or nearly so, of the specificity of either the host or donor. The agglutinating factors of the blood are at least analogous to the intracellular factors released by crushing the metanephric tissue. Both have lethal effects on the host, but are potent only when, and if, they gain entrance into the blood stream.

#### SUMMARY

1. Metanephric tissue from duck and chick embryos of comparable ages, ground in a mortar and collected by centrifuging, was implanted on the chorio-allantoic membrane of the chick and duck respectively.

2. The implantation of the macerated duck tissue had no significant effects on the chick hosts until the donor embryos approached the age of hatching, at which time it caused death within 48 hours. The average mortality rate following the implantation of tissue from donors of 24 days' incubation, or younger, was 27.9 per cent. When the tissue was obtained from donors of 27 days' incubation, the percentage of deaths among the hosts increased to 59.2 per cent, and when obtained from donors of 28 days' incubation (time of hatching), it increased to 87.7 per cent. Macerated tissue from donors older than 28 days' incubation consistently killed the hosts, the mortality rate being 100.0 per cent.

3. The reciprocal relation, i.e., chick-on-duck, showed no significant increases in mortality rate comparable to that demonstrated in the duck-on-chick implantations.

4. Death of the chick hosts following the implantation of macerated duck tissue resulted from an apparent agglutination of the blood cells by intracellular substances which were released from the cells by maceration.

5. Comparing the effects of the implantation of macerated tissue with those of the intact, it was concluded that the transplantability of a tissue, as manifested by the nature and intensity of the local inflammation reaction incited by metabolic toxins given off by the transplants of intact kidney tissue, is independent of intracellular substances which may be responsible for species specificity, and which are released from the cell only by crushing.

6. Attention was called to the fact that the local inflammation reaction incited by intact tissue transplants can be modified in several ways, so that it can be used only with considerable care in an analysis of problems pertaining to the development of species specificity.

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